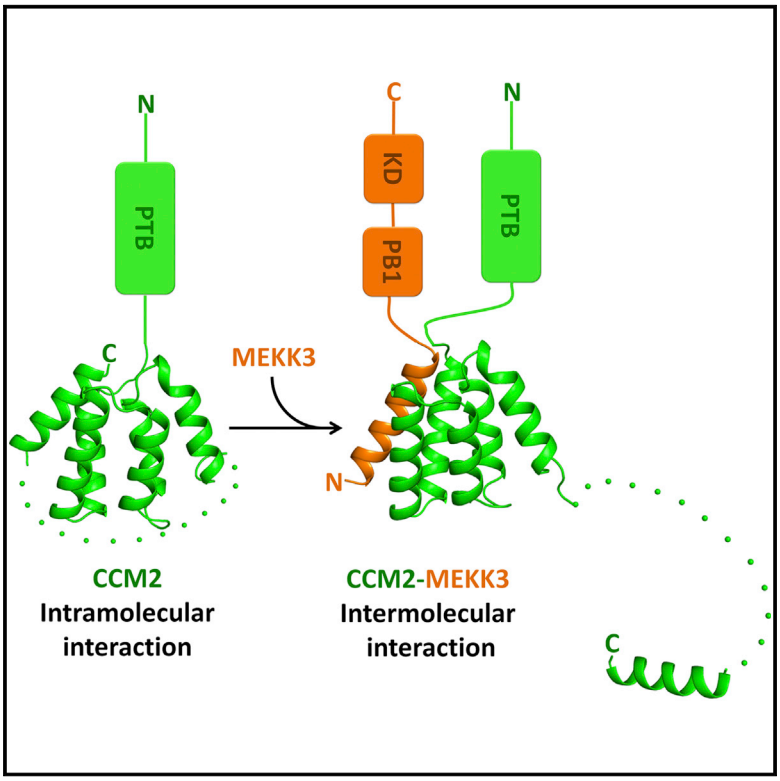
Article

Structure

Structural Insights into the Molecular Recognition between Cerebral Cavernous Malformation 2 and Mitogen-Activated Protein Kinase Kinase Kinase 3

Graphical Abstract



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In Brief

CCM2 functions as an adaptor protein that mediates the activation of MEKK3 signaling in response to osmotic stress, or negatively regulates MEKK3 signaling, which is important for normal cardiovascular development. Wang et al. reveal the structural basis governing the molecular recognition between CCM2 and MEKK3.

Highlights

- CCM2ct assembles into a global six-helix domain by intramolecular interaction
- CCM2ct intramolecular interaction is weak
- MEKK3-n_{helix} is the crucial structural element for CCM2ct binding
- The binding of CCM2ct to MEKK3-n_{helix} resembles CCM2ct intramolecular interaction

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Structural Insights into the Molecular Recognition between Cerebral Cavernous Malformation 2 and Mitogen-Activated Protein Kinase Kinase Kinase 3

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SUMMARY

Cerebral cavernous malformation 2 (CCM2) functions as an adaptor protein implicated in various biological processes. By interacting with the mitogen-activated protein kinase MEKK3, CCM2 either mediates the activation of MEKK3 signaling in response to osmotic stress or negatively regulates MEKK3 signaling, which is important for normal cardiovascular development. However, the molecular basis governing CCM2-MEKK3 interaction is largely unknown. Here we report the crystal structure of the CCM2 C-terminal part (CCM2ct) containing both the five-helix domain ($CCM2ct_S$) and the following C-terminal tail. The end of the C-terminal tail forms an isolated helix, which interacts intramolecularly with $CCM2ct_S$. By biochemical studies we identified the N-terminal amphiphilic helix of MEKK3 (MEKK3-n_{helix}) as the essential structural element for CCM2ct binding. We further determined the crystal structure of $CCM2ct_S$-MEKK3-n_{helix} complex, in which MEKK3-n_{helix} binds to the same site of $CCM2ct_S$ for CCM2ct intramolecular interaction. These findings build a structural framework for understanding CCM2ct-MEKK3 molecular recognition.

INTRODUCTION

Adaptor proteins are an emerging group of proteins that function as essential components of cellular signal transduction involved in gene expression, protein synthesis and quality control, cell metabolism, intracellular trafficking, cytoskeleton maintenance and rearrangement, cellular membrane dynamics, stress and immune response (Good et al., 2011; Pan et al., 2012). In cellular signal cascades, adaptor proteins evolve unique protein-protein and protein-ligand interaction modules to recruit their binding partners (Bhattacharyya et al., 2006; Flynn, 2001). Exploring the structural basis for adaptor-partner recognition could provide in-depth understanding of the complicated functions of related signal pathways in the cell.

Cerebral cavernous malformation 2 (CCM2) was initially characterized as a critical adaptor protein for mitogen-activated protein kinase (MAPK) cascade in response to osmotic stress. By bridging the upstream kinases MAPK/ERK kinase kinase 3 (MEKK3) and MAPK/ERK kinase 3 (MEK3) in the p38 MAPK phospho-relay pathway, CCM2 mediates the phosphorylation and ensuing activation of MEK3 by MEKK3, in turn activating downstream p38 MAPK and regulating cytoskeletal architecture (Uhlik et al., 2003). The gene encoding CCM2 has been identified as the second CCM-related gene (Denier et al., 2004; Liquori et al., 2003, 2007). Loss-of-function mutations in CCM2 result in human cerebral cavernous malformation, a common vascular lesion of the CNS that leads to headaches, seizures, stroke, and intracranial hemorrhage (Labauge et al., 2007). By physically interacting with the first CCM-related gene product KRIT1 (Krev1/Rap1A Interaction Trapped 1, also known as CCM1), CCM2 is coupled to transmembrane receptor heart of glass 1 (HEG1) signaling, which regulates the endothelial cell junction and maintains the vessel integrity (Kleaveland et al., 2009) and is involved in the signal pathway that inhibits small GTPase RhoA and its effector Rho kinase, consequently limiting actin stress fibers and vascular permeability (Borikova et al., 2010; Crose et al., 2009; Stockton et al., 2010; Whitehead et al., 2009). Moreover, CCM2 can organize a large CCM signal complex via association with both Kirt1 and the third CCM-related gene product PDCD10 (programmed cell death 10, also known as CCM3) (Faurobert and Albiges-Rizo, 2010; Hilder et al., 2007; Voss et al., 2007; Zawistowski et al., 2005), which regulates vascular stability and growth dynamically (Rosen et al., 2013; Zheng et al., 2012). Full-length human CCM2 comprises 444 residues and adopts a two-domain architecture. The N-terminal part of CCM2 contains a canonical phosphotyrosine-binding (PTB) domain, which recognizes the NPxY/F (where x is any residue) motifs of Krit1 to perform related physiological functions (Hilder et al., 2007; Zawistowski et al., 2005). In neuroblastoma or medulloblastoma cells, the N-terminal PTB domain of CCM2 has been shown to interact with the juxtamembrane region of receptor tyrosine kinase TrkA, and the C-terminal part of CCM2 links to cell death by an unknown mechanism (Harel et al., 2009). Further studies indicated that germinal center kinase class III kinase STK25 is part of TrkA-CCM2-dependent death in medulloblastoma cells through CCM2-CCM3-STK25 interactions (Costa et al., 2012).